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Microbial Mercury Transformation in Anoxic Freshwater Sediments under Iron-Reducing and Other Electron-Accepting Conditions

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Potential rates of microbial methylation of inorganic mercury (added as $HgCl_2$) and degradation of methyl mercury (MeHg) (added as CH_3HgCl) were investigated in anoxic sediments from the Mobile Alabama River Basin (MARB) dominated by different terminal electron-accepting processes (TEAPs). Potential rates of methylation were comparable under methanogenic and sulfate-reducing conditions but suppressed under iron-reducing conditions, in slurries of freshwater wetland sediment. In contrast, MeHg degradation rates were similar under all three TEAPs. Microbial Hg methylation and MeHg degradation were also investigated in surface sediment from three riverine sites, two of which had iron reduction and one sulfate reduction, as the dominant TEAP (as determined by ^{14}C-acetate metabolism and other biogeochemical measurements). Methylation was active in sulfate-reducing sediments of a tributary creek and suppressed in iron-reducing, sandy sediments from the open river, whereas MeHg degradation was active at all three sites. Although iron-reducing conditions often suppressed methylation, some methylation activity was observed in two out of three replicates from iron-reducing sediments collected near a dam. Given that MeHg degradation was consistently observed under all TEAPs, our results suggest that the net flux of MeHg from iron-reducing surface sediments may be suppressed (due to inhibition of gross MeHg production) compared to sediments supporting other TEAPs.

Introduction

Human exposure to mercury through consumption of contaminated fish is an increasing environmental concern in the southeastern United States, where high mercury (Hg) burdens have been measured in predatory fish from several rivers (*1*, *2*). The accumulation of methyl mercury (MeHg) in aquatic food chains depends partly on the net rate of microbial Hg methylation in sediments. Sulfate reducing bacteria (SRB) are generally thought to be the primary methylators of inorganic Hg in aquatic sediments (*3*−*5*), whereas a more diverse group of microbes are able to degrade MeHg. Microbial MeHg degradation or demethylation, catalyzed by both gram negative and positive prokaryotes, can proceed through reductive as well as oxidative pathways (see ref *6* and references therein).

Although Hg methylation and MeHg degradation have been investigated in a variety of sedimentary environments under sulfate-reducing and methanogenic conditions, little is known about the capacity for microbial Hg transformation in sediments dominated by other terminal electron accepting processes (TEAPs). Several studies have examined microbial Hg transformation in sediments amended with other potential electron acceptors such as nitrate, Fe(III), and Mn(IV) (*7*−*10*), but these studies did not determine whether these substrates were utilized as electron acceptors by microorganisms in the sediment.

We are currently investigating how hydrology, land use, and biogeochemical conditions influence Hg behavior in the Mobile-Alabama River Basin (MARB) in Alabama, U.S.A. The MARB contains many iron-rich upland soils and wetland sediments (*11*) and is impacted by a variety of different land uses, some of which elevate sulfate levels in certain areas. In this study, we investigated how different terminal electron accepting processes, in particular microbial iron reduction, affected potential rates of microbial Hg methylation and MeHg degradation in sediments from a mineral-rich wetland, an impounded river, and a tributary creek in the MARB. The goal of the work was to gain insight into how biogeochemical conditions in MARB sediments may affect their potential as net sources of MeHg to overlying waters and biota.

Experimental Section

Wetland Sediments. Sediments for initial microbial Hg transformation experiments and manipulative TEAP determinations were collected from a small (<1 ha), shallow (<1 m) freshwater wetland in the Black Warrior drainage of the MARB (Figure 1). Surface sediment (0−5 cm) was collected using a polyethylene scoop, transferred into 500 mL glass jars filled to capacity, and used immediately in experiments.

Riverine Sediments. Sediments were collected from three sites (dam, open river, and tributary creek) in the Demopolis Pool in west-central AL and analyzed for their dominant TEAP, geochemical characteristics, and potential Hg methylation/MeHg degradation rates. The Demopolis Pool, located in the Coastal Plain physiographic province of the MARB, is formed by a navigational lock and dam on the Lower Tombigbee River ~3 river miles downstream of the confluence of the Black Warrior and Upper Tombigbee Rivers (Figure 1). The headwaters of the Black Warrior basin drain the Appalachian Plateau province, containing 90% of all coal and gas production in AL, which contribute elevated levels of sulfate (3−10 times the concentration in Upper Tombigbee) in the Black Warrior and Lower Tombigbee main stem. The dam site (12.2 m depth) was sampled 0.5 miles upstream of the dam. The open river site (13.7 m depth) was located in the central channel of the Black Warrior River ca. 10 river miles upstream of the dam. The tributary creek site (French Creek) (2 m depth) drains ca. 3700 hectares (61% agricultural land use) of the Black Warrior River basin. Sulfate concentrations at the river, dam, and creek sites were 685, 470, and 519 μM, respectively, at the time of collection. Sediments cores (9 cm i.d.) with overlying water were collected in late summer 2001 by divers and placed in coolers until return to the laboratory. Sediments from different sites were comprised of light brown sand and silt (open river); compact light brown oxidized silt/clay (dam); and unconsolidated reduced, fine

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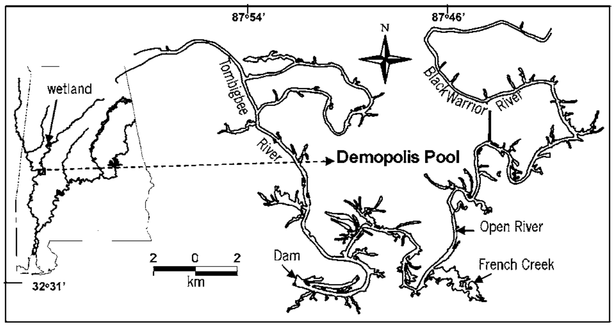

FIGURE 1. Map of Demopolis Pool in relation to MARB rivers in Alabama, showing sampling locations.

black silt (creek). Sediment processing was completed within 36 h of collection.

Wetland Sediment Methylation/MeHg Degradation Experiments. Potential Hg methylation and MeHg degradation assays were conducted according to the method of Pak and Bartha (*12*). This method involves the use of cold spikes of inorganic Hg and MeHg and has been employed successfully in other recent studies of microbial Hg transformation (*5, 13−15*). The potential toxicity of the Hg species concentrations used in these studies was assessed previously with sulfate-reducing wetland sediments and found to have no effect on rates of SR (unpublished data), although effects on other TEAPs was not assessed.

Surficial wetland sediments were wet-sieved (1 mm mesh), mixed with deionized water (1:1; vol/vol), bubbled with N_2:CO_2 (95%:5%) for 1 h, capped, and incubated in dark for several days to deplete iron(III) oxides (0.5 M HCl-extractable fraction). A portion of the reduced slurry was then oxidized to produce iron(III) oxide-rich sediment for the iron-reducing treatments, as described in Roden and Wetzel (*11*). The pH of the oxidized slurry was adjusted to ca. 6.5 with 1 M NaOH prior to being rendered anoxic by bubbling with N_2:CO_2 (90%:10%). Slurries were amended with either 1 ppm (1 μg/mL slurry) $HgCl_2$ for Hg Methylation (M) experiments or 0.06 ppm CH_3HgCl for MeHg Degradation (D) experiments and incubated for 9 d under N_2:CO_2 (90%:10%) at 22 °C. Iron-reducing treatments consisted of triplicate 100-mL bottles with 60 mL of oxidized slurry and a 3-mL inoculum of reduced slurry. Sulfate-reducing and methanogenic treatments consisted of triplicate bottles with 63 mL of reduced slurry, with the former amended with a small quantity of sterile, anaerobic Na_2SO_4 in order to obtain a starting sulfate concentration of ca. 200 μM (approximately equal to the in situ sulfate concentration in the wetland surface water). Sulfate was monitored periodically in the sulfate-reducing sediments and replenished to the starting concentration. Methanogenic treatments received no additions of electron acceptors. Homogeneous slurry subsamples were withdrawn periodically with sterile, N_2-flushed syringes to monitor changes in MeHg and sulfate concentration, pH, and iron redox speciation. Separate experiments were conducted with autoclaved wetland sediment incubated under all three TEAPs using similar doses of $HgCl_2$ or CH_3HgCl to assess the potential for abiotic production or loss of MeHg.

Riverine Sediment M/D Studies. Sediment cores were sectioned inside an anaerobic chamber with a N_2:H_2 (98%:2%) atmosphere. For the dam and creek sites, the 0−2 and 2−4 cm depth horizons were collected. For the sandy open river cores, which displayed no obvious redox stratigraphy, only one depth horizon (0−6 cm) was collected. Corresponding sediment horizons from 1 to 3 cores were combined, homogenized, and apportioned into replicate serum vials (2−3 mL sediment in 10-mL vials). Vials were capped with thick rubber stoppers, removed from the chamber, and purged (via incurrent and excurrent needles) for 15 min with N_2 to remove residual H_2 from the vial headspace. Individual vials were then dosed with either $HgCl_2$ or CH_3HgCl to a final concentration of 1 or 0.06 ppm, respectively. In some instances, either inefficient extraction or variation in the concentration of MeHg that was added resulted in measured starting concentrations that were below 0.06 μg mL^{-1}. Vials were incubated statically for 6 d in the dark at 24 °C. At periodic intervals, three vials were sacrificed for MeHg analysis by the addition of 10% (vol/vol) 1 N HCl (Optima) through the stopper and stored at −15 °C prior to extraction and analysis.

Determination of the Dominant TEAP in Wetland and Riverine Sediments. Wetland sediment slurries (prepared as described above) expressing (presumably) either iron reduction, sulfate reduction (SR) (after amendment with 2 mM sulfate), or methanogenesis as the dominant TEAP were used to verify the applicability of the ^{14}C-acetate oxidation method (*16*) to determine the dominant TEAP in MARB sediments. Briefly, this method involves the addition of 2-^{14}C-acetate to sediments, followed by short-term incubation with and without the addition of molybdate, a specific inhibitor of SRB. After incubation, the relative abundance of $^{14}CO_2$ and $^{14}CH_4$ produced during ^{14}C-acetate metabolism is determined. Production of $^{14}CH_4$ indicates methanogenesis, while $^{14}CO_2$ production signals nonmethanogenic metabolism. The extent to which molybdate inhibits $^{14}CO_2$ production provides information on the relative importance of SRB vs other nonmethanogenic organisms (e.g. nitrate-, Mn(IV)-, or Fe(III)-reducers) in acetate turnover. Since nitrate was not detected in the pore water of these sediments, and the abundance of reactive Mn was typically 10−100-fold lower than reactive Fe (as determined by 0.25 M HCl/0.25 M $NH_2OH \cdot HCl$ extraction and ICP analysis), $^{14}CO_2$ production not inhibited by molybdate was attributed to the activity of dissimilatory iron-reducing bacteria. The purpose of the preliminary experiments with the wetland sediment was to confirm that the ^{14}C-acetate technique accurately reflected the dominant TEAP as revealed by independent geochemical measurements (i.e. Fe(III) and sulfate consumption, methane production). Since this was the case (see Results), we applied the technique to determine the dominant TEAP in the riverine sediment samples.

For all ^{14}C-acetate metabolism experiments, triplicate sediment subsamples (2 mL) were transferred to 5 mL serum vials inside the anaerobic chamber. The vials were then removed from the chamber and flushed for 15 min with N_2 to remove H_2 from the headspace. Molybdate-treated sediments were amended with 2 mM Na_2MoO_4 (from a 1 M sterile, anaerobic stock solution) 1 h prior to addition of ^{14}C-acetate. All vials were spiked with 0.14 μCi of 2-^{14}C-acetate (Amersham, ca. 50 mCi mmol^{-1}) and incubated for 1 h. Activity was terminated by addition of 0.5 mL of 1 N HCl. The amount of $^{14}CO_2$ and $^{14}CH_4$ produced was determined by gas proportional counting as described below.

Studies of ^{14}C-acetate metabolism in riverine sediments were initiated 0, 3, and 6 d after the start of the M/D assays in order to monitor potential changes in the dominant TEAP during the course of the microbial Hg transformation experiments. In general, the amount of ^{14}C-acetate converted to $^{14}CO_2$ and/or $^{14}CH_4$ was greatest at day zero and decreased thereafter. However, it is the relative proportion of $^{14}CO_2$ and $^{14}CH_4$ end products that indicates the dominant TEAP rather than the total mass of ^{14}C-acetate mineralized. Hence, only the relative proportion of ^{14}C end products is presented in the results.

Sulfate Reduction Rate Assays. Triplicate subsamples of riverine sediment were prepared as described above and amended with 1 μCi of $^{35}S{-}SO_4^{2-}$. After incubation for 0.5−1 h (or 0 h for time-zero controls), activity was terminated by

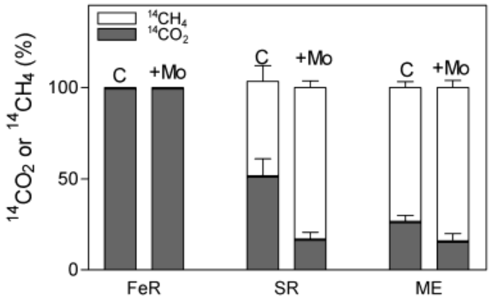

FIGURE 2. Percentage of $^{14}CO_2$ or $^{14}CH_4$ produced from ^{14}C-acetate incubated in iron-reducing (FeR), sulfate-reducing (SR), or methanogenic (ME) wetland sediments in live control (C) (i.e. no molybdate) and + molybdate (+Mo) treatments. Each determination is the mean of 3 replicates ± 1 SD.

injection of 0.4 mL of 10% zinc acetate, and the samples were frozen. The quantity of ^{35}S-labeled reduced sulfur produced during $^{35}SO_4^{2-}$ reduction was determined using the single step Cr(II) reduction method of Fossing and Jorgensen (*17*). Less than 25% of the added $^{35}S-SO_4^{2-}$ was reduced during the incubations, and the ^{35}S-labeled reduced S recovered was corrected for the activity recovered from time zero controls.

Analytical Techniques. MeHg was determined using a method adapted from Bloom (*18*). MeHg was released from sample matrix by alkaline alcohol digestion, followed by ethylation, Tenax trapping, GC-separation, thermodecomposition, and detection by cold vapor atomic fluorescence spectrometry (CV-AFS). Samples for total-Hg (THg) determination were first digested, in acid-cleaned flasks, with a 7:3 mixture of HNO_3/H_2SO_4 (*19*, *20*) and analyzed by $SnCl_2$-reduction technique with detection by CV-AFS (*21*). For both

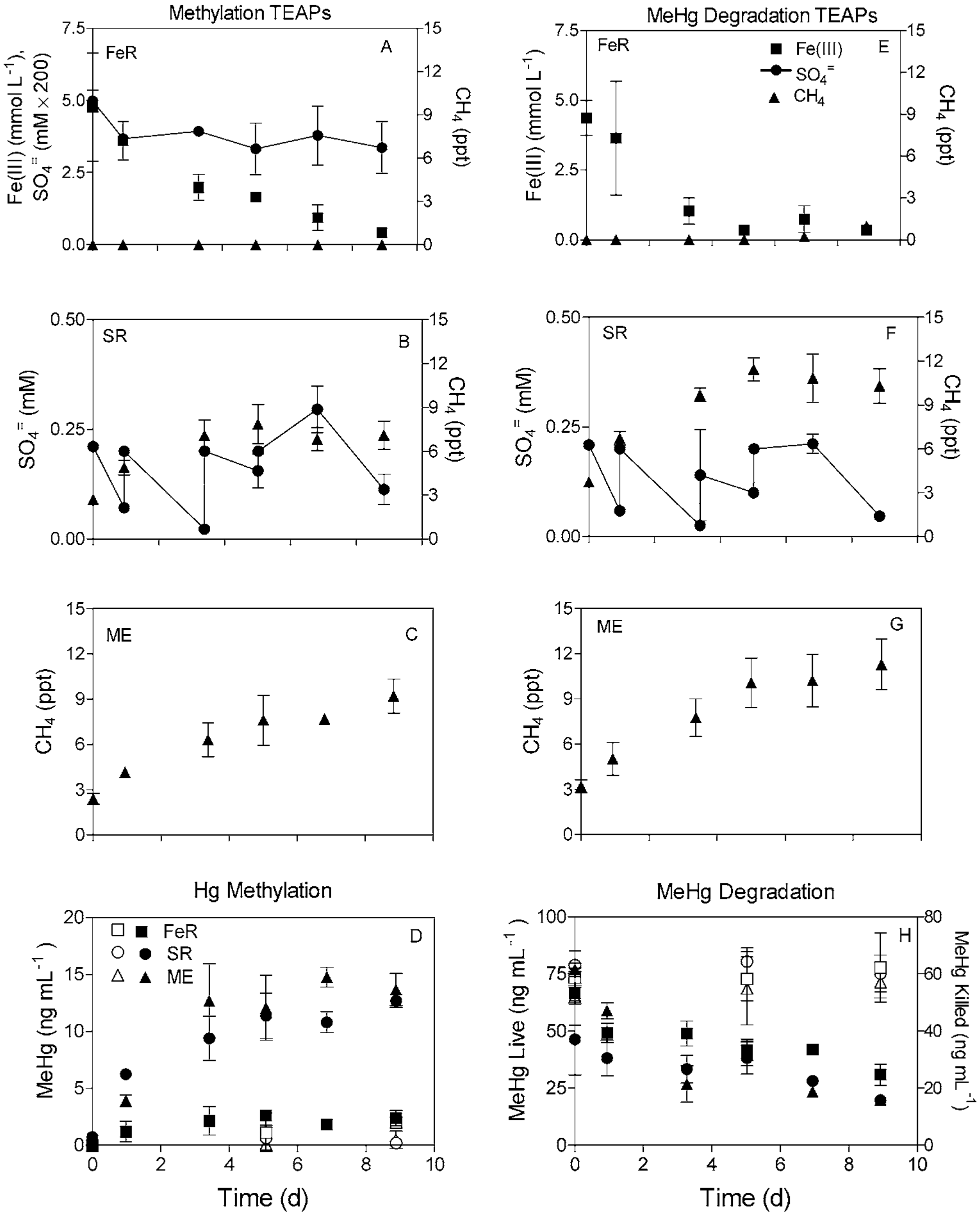

FIGURE 3. Consumption of electron acceptors and methane production in iron reducing (FeR: A,E), sulfate reducing (SR: B,F), and methanogenic (ME: C,G) wetland sediment treatments in corresponding Hg methylation (D) and MeHg degradation (H) assays, respectively. (■): Fe(III) concentration (A,E) and loss or production of MeHg in live FeR treatments. (●): SO_4^{2-} concentration (A,B,F) and loss or production of MeHg in live SR treatments (D,H). (▲): CH_4 concentration (A−C; E−G) and loss or production of MeHg in live ME treatments (D,H). Panels D and E: Closed symbols: live incubations; open symbols: killed controls. Each determination is the mean of 3 replicates ± 1 SD.

TABLE 1. Sediment Biogeochemical Parameters, Porewater Solute Concentrations, and ^{35}S-Sulfate Reduction Rates (SRR) for MARB Riverine Sites[b]

depth	porosity	LOI, %	Fe(II), μmol cm^{-3}	Fe(III),[a] μmol cm^{-3}	SRR, μmol cm^{-3} d^{-1}	SO_4^{2-}, μM	HS^{1-}, μM	THg, ng g^{-1} d.w.	MeHg ng g^{-1} d.w.
Creek									
0–2 cm	0.86	8.3	44	0.40	1250	332	1.7	260	0.51
2–4 cm	0.83	8.1	55	0.10	382	53	2.1	204	0.64
Dam									
0–2 cm	0.67	3.8	20	62	5.2	846	0.6	201	0.40
2–4 cm	0.64	4.4	43	55	22	1150	ND	193	0.35
Open River									
0–6 cm	0.40	0.2	3.4	14	0.11	619	ND	67	0.13

[a] 0.5 M HCl-extractable fraction. [b] ND: not detected; LOI: loss on ignition.

MeHg and THg, in addition to reagent blanks and liquid standard solutions, a certified reference material (IAEA-405, estuarine sediments containing an average of 0.81 mg Kg $^{-1}$ THg and 5.49 ng g^{-1} of MeHg) was run with all digestions/analyses. The percent recovery on the IAEA-405 averaged 95 ± 11% ($n = 10$) and 93 ± 8% ($n = 10$) for THg and MeHg, respectively.

Porewater samples for analysis of dissolved sulfate, nitrate, sulfide, and Fe(II) were collected via centrifugation inside the anaerobic chamber and passed through a 0.2 μm filter. Sulfate and nitrate were measured by ion chromatography (Dionex Model DX100). Sulfide was determined by a modified version of the Cline (*22*) method. Dissolved Fe(II) was determined using ferrozine (*23*).

The concentrations of amorphous Fe(III) oxide and solid-phase Fe(II) in wetland slurries and river sediments were determined via 0.5 M HCl and ferrozine analysis (*24*). Methane production during the wetland sediment incubations was measured by gas chromatography with flame ionization detection (*11*). Sediment organic matter (loss on ignition or LOI) was determined by difference in weight of dried and combusted (500 °C) sediment material.

The $^{14}CO_2$ and $^{14}CH_4$ produced during ^{14}C-acetate metabolism were separated on a 6′ × 1/8″SS Carbosphere 80/100 column (Alltech) in a Shimadzu GC-8A gas chromatograph with He as the carrier at a flow rate of 27 mL min^{-1}. Column and injector/detector temperatures were 80 and 120 °C, respectively. $^{14}CO_2$ and $^{14}CH_4$ were detected on a GC-RAM gas proportional counter (IN/US Systems, FL), following combustion of $^{14}CH_4$ at 750 °C, using propane as the quench gas (flow 6 mL min^{-1}). Peaks were identified by external standards and areas quantified by WinFlow software (IN/US). The efficiency of the radioactive gas counting was determined by analysis of acidified ^{14}C-bicarbonate standards.

Results and Discussion

Wetland Sediment TEAPs. Wetland sediment slurries were used to assess whether the proportion of ^{14}C-acetate metabolized to $^{14}CO_2$ vs $^{14}CH_4$ in the presence and absence of molybdate (a specific inhibitor of SR) could be used as a valid indicator of the dominant TEAP in MARB sediments. Results from these experiments showed clear differences in the proportion of $^{14}CO_2$ and $^{14}CH_4$ formed from ^{14}C-acetate under the three imposed TEAP conditions (Figure 2). Molybdate had no effect on $^{14}CO_2$ production in the iron-reducing slurries. In the sulfate-reducing slurries, ca. 50% of the metabolized ^{14}C-acetate was recovered as $^{14}CO_2$, and the addition of molybdate reduced the amount of $^{14}CO_2$ and increased the amount of $^{14}CH_4$ formed ca. 2-fold. In the methanogenic treatments, $^{14}CH_4$ accounted for ca. 80% of ^{14}C-acetate metabolism, and molybdate had no significant effect on the proportion of end products.

Changes in the concentrations of Fe(III) (Figure 3A,E), sulfate (Figure 3B,F), and methane (Figure 3C,G) in the wetland sediment slurries confirmed inferences based on ^{14}C-acetate metabolism regarding the predominant TEAPs in the different treatments. Methane production and sulfate consumption were almost completely suppressed in the iron-reducing slurries (Figure 3A,E). In contrast, sulfate was repeatedly reduced throughout the assay period in the sulfate-reducing treatments (Figure 3B,F). The production of substantial quantities of methane in these sediments is consistent with the finding that $^{14}CH_4$ production accounted for ca. 50% of total ^{14}C-acetate metabolism in these treatments (Figure 2). Finally, rates of methane production in the Fe(III)- and sulfate-free methanogenic slurries (Figure 3C,G) were equal to or greater than those in the sulfate-reducing slurries (Figure 3B,F), which is consistent with the inference based on ^{14}C-acetate metabolism that methanogenesis was the dominant TEAP in these sediments. Collectively, these results validate our use of ^{14}C-acetate metabolism as an indicator of the predominant TEAP in the MARB sediment microbial Hg transformations studies described below.

Wetland M/D Experiments. Hg methylation rates were similar under sulfate-reducing and methanogenic conditions in wetland sediment slurries (Figure 3D). In both treatments, MeHg accumulated linearly during the first 3–4 d of incubation, after which it leveled off at values equal to ca. 1.5% of the added $HgCl_2$. Hg methylation was suppressed under iron-reducing conditions, with final MeHg levels close to those observed in killed controls (Figure 3D). MeHg degradation rates, however, were similar among under all three TEAPs and resulted in a steady loss of over 50% of the added MeHg (Figure 3H). Experiments with autoclaved wetland sediments showed negligible loss of added MeHg during 9 days of incubation (Figure 3H); thus, the loss of MeHg in live incubations can be attributed to biological activity.

Riverine Sediment TEAPs. The observation of active SR in both sediment depths (Table 1), and the suppressive effect of molybdate on $^{14}CO_2$ production (Figure 4A,B), indicated that SR was the dominant TEAP in surface sediments at the tributary creek site, at least through day 3 of the 6-d M/D assay period. By day 6, methanogenesis appeared to play some role in electron flow in sediment from both depths. The apparent lack of iron reduction activity at this site is consistent with the very low abundance of reactive Fe(III) (Table 1).

Despite the greater availability of sulfate in sediments at the river and dam sites (Table 1), ^{14}C-acetate metabolism indicated that iron reduction was the dominant TEAP at both sites (Figure 4C–E): virtually all metabolized ^{14}C-acetate was recovered as $^{14}CO_2$, and molybdate had no effect on the $^{14}CO_2$ production. This conclusion is supported by the presence of substantial quantities of reactive Fe(III) and the very low

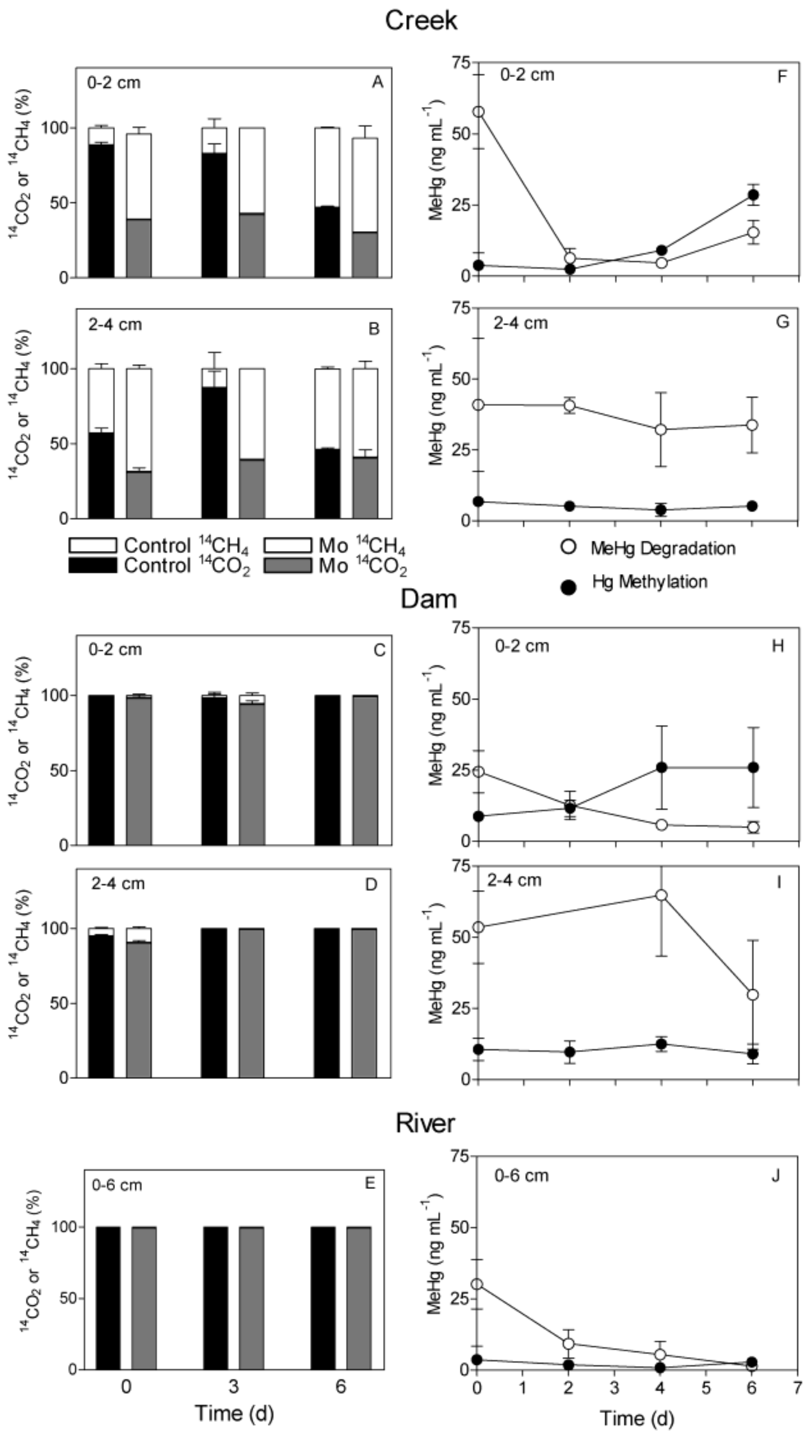

FIGURE 4. Production of $^{14}CO_2$ and/or $^{14}CH_4$ from ^{14}C-acetate (A−E) in riverine sediments and corresponding Hg methylation and MeHg degradation potentials (F−J) from creek, dam, and river sites. "Control $^{14}CH_4$ and $^{14}CO_2$" in plots A−E refer to live incubations without molybdate. Each determination is the mean of 3 replicates ± 1 SD.

measured rates of SR (Table 1).

Riverine Sediment M/D. Microbial transformation of added Hg or MeHg was generally active in surface sediments at all three riverine sites (Figure 4F,H,J). If microbial Hg transformations were active in the lower depths (2−4 cm) of the creek (Figure 4G) or dam (Figure 4I) sites, this activity was less than the variability among replicates and could not be determined with our methods. The observation of greater Hg transformation activities in surface sediments is consistent with results from other systems (*25*, *26*).

Hg methylation was observed in surface sediments from both the sulfate-reducing creek site (Figure 4F) and in two out of three replicates at the iron-reducing dam site (up to 34 ng mL^{-1}) (Figure 4H). However, two-way ANOVA revealed no significant difference ($p > 0.1$) between the means of the starting and final MeHg concentrations in the methylation assays at the dam site. No methylation activity was observed in the iron-reducing open river sediments (Figure 4J).

Consistent with the results from the wetland slurry experiments, MeHg degradation was active in surface sediments from all three sites, regardless of the dominant TEAP (Figure 4F,H,J). Rapid initial rates of MeHg degradation were observed in the creek sediments (Figure 4F), after which some methylation of recently degraded MeHg may have taken place, thereby accounting for the increase in MeHg observed at the end of the incubation period. MeHg degradation in the dam and open river sediments was more gradual and took place over the entire assay period.

Influence of TEAPs on Microbial Hg Transformation in MARB Sediments. Hg methylation rates were similar under sulfate-reducing and methanogenic conditions in the wetland sediment slurries (Figure 3D). Similar results were reported by Pak and Bartha (*12*) for anoxic lake sediments and may be explained by Hg methylation by fermentative SRB functioning in syntrophic association with methanogens (*27*). Furthermore, given that methylation potentials in some replicates of the iron reducing dam site (Figure 4H) were comparable to those in the sulfate-reducing creek site (Figure 4F), no relationship between SR and methylation activities was evident in the MARB sediments examined in this study. In contrast, a positive relationship between SR and Hg methylation rates was observed in some marine (*13*, *14*, *28*) and freshwater sediments (*4*), while Gilmour and colleagues (*8*) found an inverse relationship in Everglades sediments, where high sulfide concentrations (up to 300 μM) accompanying rapid SR rates decreased the bioavailability for Hg for methylation. Although sulfide concentrations were uniformly low in the sediments examined here (Table 1) and likely did not affect Hg methylation, the complex influence of sulfur biogeochemistry on Hg speciation and bioavailability in aquatic sediments (*29*) does not allow us to draw firm conclusions regarding the lack of correlation between SR activity and Hg methylation in MARB sediments. Nevertheless, the important point of our findings is that they confirm the results of earlier studies (*12*, *27*, *30*, *31*) demonstrating that a lack of SR activity does not necessarily preclude methylation.

To our knowledge, the studies reported here represent the first assessment of the potential for Hg methylation in sediments in which iron reduction was the dominant TEAP. Hg methylation was clearly suppressed in the iron-reducing wetland sediment slurries compared to sulfate-reducing and methanogenic treatments (Figure 3D). In addition, Hg methylation activity was mostly (but not always) suppressed in riverine sediments in which ^{14}C-acetate metabolism indicated that iron reduction was the dominant TEAP (Figure 4). It is not clear whether the observed suppression of Hg methylation under iron-reducing conditions was the result of lower bioavailability of Hg (e.g. as a result of Hg sorption to iron(III) oxide surfaces) or the inability of Hg methylators (e.g. SRB) to effectively compete for substrates with iron-reducers.

Other research has shown that iron oxides efficiently scavenge both Hg (*10*, *32*) and MeHg (*33*), presumably rendering them less available for microbial transformation. Despite these findings, results from studies examining microbial transformation of Hg species in the presence of Fe(III) have been mixed. Jackson (*10*) observed no effect of the binding/complexation of Hg by added iron(III) oxides on Hg methylation or MeHg degradation activity in some lake sediments, whereas inhibition of both processes occurred in other sediments. Gilmour et al. (*8*) found no effect of soluble Fe(III) (ferric citrate) on Hg methylation in sulfate-reducing Everglades sediments. It is unclear from these studies, however, whether the Fe(III) added to these sediments was available for and subject to microbial reduction. For example, the presence of abundant ferrous iron in reduced sediments may have led to rapid coating of iron(III) oxide surfaces with sorbed Fe(II), thereby rendering them unavailable for microbial reduction (*34*). In addition, soluble Fe(III) added to sulfidic freshwater sediments may have been subject to rapid chemical reduction by sulfide, thereby preventing its utilization by iron-reducing bacteria. Our studies provide the first clear documentation of the potential for Hg methylation in sediments in which iron reduction was shown to be a dominant TEAP, although the mechanism(s) whereby the presence of iron-reducing conditions suppressed methylation cannot be determined with available data. Regardless of the mechanism for Fe(III) oxide suppression of Hg methylation, our results have important implications for proposed linkage between sulfate abundance and Hg methylation in some freshwater sediments (*4*, *35*), particularly where sulfide concentrations are low (*36*): methylation may not be active even in sediments containing abundant sulfate if iron(III) oxides are present as sorbents for otherwise bioavailable Hg or as competing terminal electron acceptors whose utilization suppresses the activity of Hg methylating microorganisms (e.g. SRB). Obviously, more research examining bioavailability and microbial transformations of Hg under iron-reducing conditions in other environments is warranted.

In contrast to the generally negative influence of iron-reducing conditions on Hg methylation, MeHg degradation was consistently observed under such conditions, at rates comparable to those observed in sulfate-reducing and methanogenic MARB wetland sediments (Figures 3H) and at variable rates in the riverine sediments (Figure 4F,H,J). These limited data suggest that the potential for net MeHg production is likely to be less in iron-reducing than in sulfate-reducing or methanogenic freshwater surface sediments. Hence, the presence of an iron(III) oxide-rich surface layer with active MeHg demethylation and suppressed Hg methylation may decrease the flux of MeHg to the overlying water column. This supposition is consistent with and may provide a mechanistic explanation for the findings of Gagnon et al. (*37*, *38*), who found that the presence of a thin surface sediment oxide layer suppressed MeHg flux from underlying anoxic marine sediments to the overlying water. Many of the aquatic sediments in the MARB sampled in other studies (Warner et al., in preparation) possessed such an iron(III) oxide-rich surface layer. If the results of our experiments are applicable to other MARB sediments and freshwater systems in general, assessment of the role of microbial iron reduction in surface sediment metabolism may be important for understanding controls on the entry of MeHg into the aquatic food chain and its bioaccumulation in higher trophic levels.

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